Thin-Layer (Planar) Chromatography

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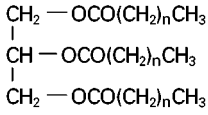
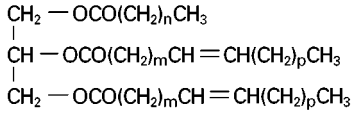
Introduction

Triglycerides (TGs) belong to the larger group of natural products called 'lipids'. A lipid is one of a wide range of natural materials that are generally based on fatty acids or closely related compounds, are insoluble in water, but soluble in organic solvents. Lipids that are solid at ambient temperature are termed 'fats' whilst those that are liquids are described as 'oils'. Lipids can be split into two groups; neutral lipids, which include acylglycerols, fatty acids, alcohols and waxes, and polar lipids, which include phospholipids and glycolipids.

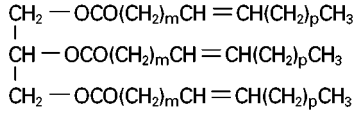
TGs make up a major part of the group of neutral lipids and are found in an extensive range of animal and vegetable fats, seed and plant oils. Lipids are present in body organs and fluids. They also find their way into many other food products, e.g. frying oils, salad dressings, margarine, butter, and various other types of spreads.



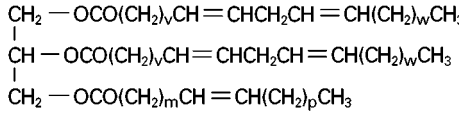
Fully saturated triacylglyceride. (*n* is usually 16 or 18)



Triacylglyceride with one 'arm' saturated and the other two unsaturated. (Values for m and p can vary, but is most commonly 7)



Triacylglyceride with all three 'arms' unsaturated



Unsaturated triacylglyceride with two 'arms' di-unsaturated and one mono-unsaturated. (v and w can vary but usually p = 7 and w = 4)

Figure 1 Structures of different types of underivatized triacylglycerides.

TGs are fully acylated derivatives of the trihydric alcohol, glycerol. Hence more accurately they should be described 'triacylglycerides', but quite often they are commonly called 'triglycerols' or 'triacylglycerols'. The structure of this group of lipids is shown in **Figure 1**. Each 'arm' of the glyceride is an ester of a fatty acid. This chain can be fully saturated or it can vary in unsaturation. Some natural triacylglycerides have the same three ester groups, e.g. tristerin (18 : 0), tripalmitin (16 : 0), triolein (18 : 1), trilinolein (18 : 2), and trilinolenin (18 : 3). More usually the fatty acid esters are different on each glycerol 'backbone' leading to many variations dependent on the number of fatty acids available and on the degree of unsaturation.

Degradation

Triacylglycerides are susceptible to hydrolysis with the resulting products being free fatty acids (FFAs), diacylglycerides (DGs), and monoacylglycerides (MGs). If the fatty acid esters are formed from unsaturated fatty acids, then the susceptibility to oxidation and hydrolytic degradation is increased. Unsaturated triacylglycerides undergo oxidative breakdown involving the formation of free radicals. This process can occur just in the presence of atmospheric oxygen at ambient temperature, although the process will be accelerated by increase in temperature. The primary products are initially allylic hydroperoxides that then undergo a series of complex reactions to form volatile compounds including aldehydes, ketones, alcohols, esters, and short chain fatty acids (see **Figure 2**).

Hydrolytic breakdown normally occurs at elevated temperatures and is often catalysed by enzymes; e.g. lipases. This degradation results in di- and monoacylglycerides and long chain fatty acids (see **Figure 3**).

Thin-Layer Chromatography

Without doubt thin-layer chromatography (TLC) is one of the simplest and most widely employed techniques in the analysis of lipids. Over the past 30 years, planar chromatography on a silica gel matrix has proved to be the most practical method of distinguishing between lipid classes including glycolipids, acylglycerols, phospholipids, sphingolipids, and ether lipids. The continued interest in improving the separation capabilities for lipids using TLC is reflected in the recently published literature.

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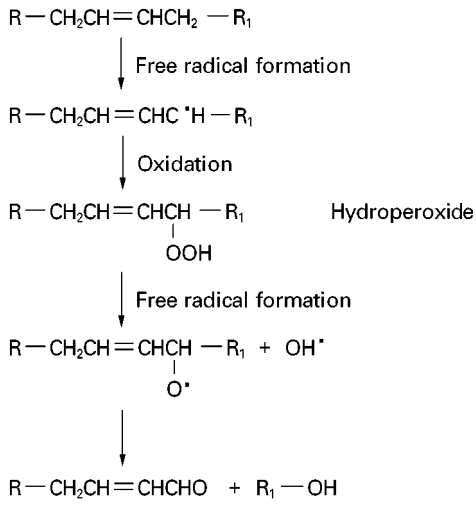

Figure 2 One possible route for autoxidation of unsaturated lipids. R = carbon chain length linked to the glyceryl backbone via a COO linkage, R_1 = a saturated carbon chain. Other degradation routes can occur and result in mixtures of aldehydes, ketones, alcohols, esters, and acids. A route to aldehydes and alcohols is shown.

Normal Phase Separations

Of all the stationary phase adsorbents available, silica gel 60 has proved to be the adsorbent of choice for the rapid separation of triacylglycerides and their

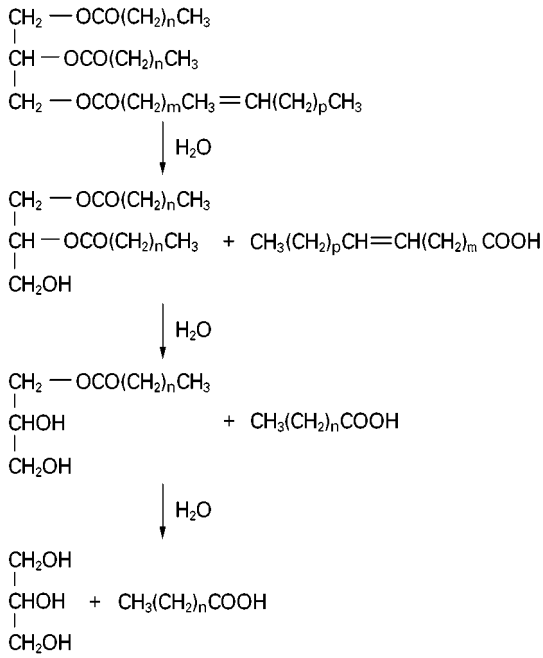

Figure 3 Hydrolytic reaction of a typical triacylglyceride. The reaction is usually catalysed by the presence of lipase. The values of n, m, and p are variable depending on the particular triacylglyceride. In the majority of cases, n = 14 (palmitic) or 16 (oleic), and m and p are 4 and 7, respectively (linoleic).

Table 1 Separation of acylglyceride classes on normal phase silica gel. Mobile phase: n-hexane–diethyl ether–acetic acid (70 : 30 : 1, v/v)

Glyceride	*R_F value (approx.)*
TG	0.70
FFA	0.45
1,2-DG	0.26
1,3-DG	0.23
MG	0.05

hydrolysis products, including any hydrolytic damage that may have occurred as a result of lipolysis. Normal phase separations enable the resolution of neutral lipids into TGs, DGs, MGs and FFA. The solvent used is normally a mixture of diethyl ether and hexane, pentane or a low boiling petroleum spirit. The ratio is in the range of 15–30% v/v diethyl ether in the saturated hydrocarbon. A modification with a small amount of formic or acetic acid (about 1%, v/v) helps to improve resolution and is necessary where any organic acids (fatty or otherwise) are suspected as being present in the sample. This aids in suppressing ionization of any FFA. The order of retention on the chromatographic layer of sample components tends to follow the expected adsorption/partition type mechanisms. The triacylglycerides, being the least polar, exhibit the least retention and hence migrate well up towards the solvent front with any sterol esters that may be present in the sample. Perhaps surprisingly these are closely followed by the fatty acids. DGs migrate much more slowly and monoglycerides show only the minimal movement from the origin. One of the interesting features of the normal phase separation of TG is the ability to clearly resolve the 1,3 and 1,2 isomers of DGs that may be present (see **Table 1**). In order to attain adequate migration of the MG from the origin, the TLC plate can be developed twice with diethyl ether to a solvent distance of 20–30 mm with intermediate drying. This enables sufficient migration of the MG from any more polar lipids on or near the origin. Following this separation step, the standard development can be carried out as before. Sometimes the TG zone on the chromatogram may appear somewhat elongated and even a partial resolution of components may be observed. This is due to the variation in the saturation and fatty acid ester chain length of the TG present.

For densitometric evaluation, silica gel 60 high performance thin-layer chromatography (HPTLC) plates can be used and samples applied using an automated band applicator. After development and detection with an appropriate reagent the chromato-

Table 2 Separation of acylglyceride classes on normal phase silica gel impregnated with 5% w/v sodium carbonate. Mobile phase: diethyl ether–n-hexane–methanol (65 : 35 : 3, v/v)

Glyceride	*R_F value (approx.)*
FFA	0.00
MG	0.18
1,3-DG	0.79
1.2-DG	0.85
TG	0.98

graphic tracks can be scanned at set wavelengths using a spectrodensitometer. Using external standards on the layer, accurate quantification of the separated components can be obtained.

To a limited extent the enzymic hydrolysis of TG in food products can be followed. Sodium carbonate-impregnated (5%, w/v) silica gel plates are used. Before the sample is applied to the layer, the enzymatic action is terminated by the addition of sodium dodecyl sulfate (SDS). The chromatogram is developed for a very short period (about 1 minute) with diethyl ether–methanol (97 : 3, v/v), which results in all the acylglycerides migrating with the solvent front and the fatty acids remaining at the origin. A modification to this solvent system; diethyl ether–n-hexane–methanol (65 : 35 : 3, v/v) results in a separation of all the various acylglycerides from the fatty acids (see **Table 2**).

Variations on the above mobile phases have been developed depending on the type of separation required and the origin of the sample. **Table 3** lists a number of solvent mixtures that have proved successful for various types of TG separations.

When lipid mixtures prove to be complex, two-dimensional systems can be helpful in resolving the large number of components. Although seldom used, there are instances where two-dimensional TLC has enabled the separation of mixed acylglycerides from steryl esters, methyl esters and fatty acids. Sample components can be resolved using a first development with n-hexane–diethyl ether (80 : 20, v/v). This is followed by plate drying and development in the second dimension at 90° to the first using a solvent mixture composed of n-hexane–diethyl ether–methanol (70 : 20 : 10, v/v). If more polar lipid components are present, then an alkaline-based solvent mixture is recommended for the development in the first dimension (chloroform–methanol–0.88 ammonia solution–water [65 : 30 : 2 : 2, v/v]) followed by an acid-based one in the second dimension (chloroform–methanol–acetic acid–water [100 : 15 : 15 : 3.5, v/v]).

Other Modifications to Normal Phase Separations

Orthoboric acid Orthoboric acid-impregnated silica gel layers are used in the TLC of TG to prevent acyl migration from the 2 to the 1 or 3 position on the glycerol backbone. The speed of migration is dependent on the acyl moiety. It is therefore important that this effect is prevented from occurring in an analysis of DG and MG. Orthoboric acid does this by weak interaction and complex formation with the free hydroxyl groups on the acylglycerides.

Precoated TLC plates can be impregnated with orthoboric acid (15% w/v) dissolved in water–methanol (25 : 75, v/v). Either dipping or spraying the plate in the solution gives satisfactory results. The plates are dried after impregnation for 30 minutes at 110°C. Separations can then be performed with methanol–chloroform (3 : 97, v/v) as solvent.

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Silver nitrate Silver nitrate or argentation TLC has been used extensively for the analysis of triacylglycerides. The reason for its popularity is that silver nitrate has a retarding effect on acylglycerides that contain unsaturated fatty acid ester moieties. The silver nitrate forms complexes with varying strength of bonding by interaction with the π double bonds. The more double bonds present, the greater the complexation and the less accessible the double bonds, the less the complexation. Hence, polyunsaturated glycerides and FFA will be more retained than their oligo-

Table 3 Solvent mixtures recommended for the separation of acylglycerides on normal phase silica gel

Acylglycerides	*Adsorbent*	*Mobile phase*
TG, DG, MG, FFA (as classes)	Silica gel 60	Diethyl ether–n-hexane–acetic acid (80 : 19 : 1, v/v)
TG, DG, MG, FFA from plasma	Silica gel 60	Toluene–diethyl ether–ethyl acetate–acetic acid (8 : 1 : 1 : 20, v/v)
Human aortic lipids including unsaturated TG	HPTLC silica gel	n-Hexane–diethyl ether–acetic acid (65 : 35 : 1, v/v)
TG, FFA, amides and cholesterol	Silica gel	Toluene–diethyl ether–ethyl acetate–acetic acid 75 : 10 : 13 : 1.2, v/v)
TG containing oxygenated fatty acid methyl esters	Silica gel 60	n-Hexane–diethyl ether (30 : 70, v/v)
TG containing epoxy and hydroxyl fatty acids	Silica gel 60	n-Hexane–diethyl ether (1 : 1, v/v)

Table 4 Solvent mixtures that have proved satisfactory for the separation of acylglycerides on silica gel impregnated with silver nitrate

Sample containing acylglycerides	*Mobile phase*
Soybean and fish oils	Diethyl ether–n-hexane (8 : 92, v/v)
Palm oil, lard, beef tallow, cocoa butter and groundnut oil	Chloroform–cyclohexane (1 : 1, v/v)
Lard and cocoa butter	Chloroform–benzene–diethyl ether (70 : 30 : 1.5, v/v)
Triacylglyceride standards	Benzene–diethyl ether (85 : 15, v/v)
Positional isomers of triacylglycerides, lard, and sunflower oil	Chloroform–methanol (99 : 1,v/v)
Orange seed oil	Petroleum ether (40–60°C)–acetone (100 : 7, v/v)

unsaturated counterparts whilst any saturated components remain unaffected. As accessibility of the double bonds also has a bearing on the degree of complexation, *cis* and *trans* isomers can be separated and acylglycerides of fatty acids that only differ in the positional location of the double bond can often be resolved.

Impregnation of silica gel 60 plates can be achieved with silver nitrate (10% w/v) dissolved in water–methanol (15 : 85 v/v). Precoated TLC and HPTLC plates are dipped in the silver nitrate solution for 10–20 s. After draining, the plates are dried in air under fume extraction and then heated for activation at 80°C for 20 minutes.

Argentation TLC has proved to be of immense importance in a number of research areas including plant-derived oils and confectionery fats. In fact, the technique has been proposed as a method for the determination of 2-oleo-1,3-disaturated triacylglycerides in cocoa butter as a part of the necessary analysis in the manufacture of chocolate. As expected, the separation of triacylglycerides follows the order of the number of double bonds with the least unsaturated being the least retained. However, if the unsaturation is in the 2-position, then there is some hindrance to the formation of the silver complex and some differentiation in the separation between the 2- and 1- or 3-position can be observed. As an example of this, it is possible to separate 2-oleo-1,3-distearin (SOS) and 3-oleo-1,2-distearin (SSO). As the interaction of the silver ion with the 2-position isomer is more sterically hindered, this is the one which is less retained on the layer and hence has the slightly higher R_F value. Of course, not only do TG vary in the amount and position of unsaturation, but also both *cis* and *trans* isomers of the same fatty acid esters occur. Examples of this are *cis*-9-octadecanoic acid (oleic acid) and *trans*-9-octadecanoic acid (elaidic acid). If any *trans* isomers are present, these are less retained than the *cis* isomers. Structurally this would be expected as the *cis* double bond is more accessible to the large silver ion, and hence complexes more readily. The general order of separation starting from the least retained and representing the fatty acid chains of the TG as 0, 1, 2, or 3 depending on the number of double bonds is: 000, 001, 011, 002, 111, 012, 112, 022, 003, 122, 013. 222, 113, 023, 123, 223, 033, 133, 233, 333.

For the common C_{18} chain, the fatty acid chains would be stearic acid (18 : 0), oleic acid (18 : 1), linoleic acid (18 : 2), and linolenic acid (18 : 3). Whilst C_{18} represents one of the most common chain lengths, shorter and longer chain lengths do occur and this increases the complexity of the problem. Palmitic acid (16 : 0) occurs more, widely naturally than stearic acid (18 : 0), being present in almost all vegetable fats, fish oils and milk fats. Fortunately for the analyst, the unsaturated versions of the C_{16} chain such as palmitoleic acid (16 : 1) are only minor components of seed oils and animal fats and only take on significant proportions in fish oils. Typical solvent mixtures used for the development of silver nitrate chromatograms are given in **Table 4**.

Both symmetrical and unsymmetrical TG are present in lard and cocoa butter and these can be separated effectively with two-dimensional argentation TLC. Unsymmetrical TG occur where the carbon chain on position 1-, 2- or 3- on the glycerol backbone vary in length. Examples of this are: POS (palmitin (16 : 0), olein (18 : 1), and stearin (18 : 0) or PPO (palmitin (16 : 0), palmitin (16 : 0), and olein (18 : 1). The separation is carried out on a dual stationary phase plate. One section of the plate is coated with a thin strip of reversed-phase silica gel, and the rest is coated with a normal phase silica gel. The sample is applied to the reversed-phase strip and the chromatogram developed using acetonitrile–acetone (80 : 20, v/v) as mobile phase. The normal-phase silica gel portion of the plate is impregnated with silver nitrate and the second dimension development then proceeds with a mobile phase composed of chloroform–benzene–diethyl ether (70 : 30 : 1.5, v/v).

Reversed-phase Separations

The resolution of TG on reversed-phase layers is usually noticeably better than that on normal-phase TLC. Although separation of acylglycerides, and FFA

Table 5 Stationary and mobile phase conditions for the separation of acylglycerides and free fatty acids on reversed-phase silica gel plates

Sample containing acylglycerides	*Stationary phase*	*Mobile phase*
Most seed oils (e.g. sunflower, olive, rapeseed oils)	HPTLC-silica gel RP18 glass plates	Dichloromethane–acetic acid–acetone (1) (20 : 40 : 50, v/v)
Most seed oils	HPTLC silica gel RP18 glass plates	Chloroform–acetonitrile–acetone (2) (20 : 40 : 50, v/v)
Most seeds oils, DG, MG, and FFA	HPTLC silica gel RP18 glass plates	Dichloromethane–ethyl acetate–methanol–acetic acid (3) (27 : 22 : 38 : 12, v/v)

into respective groups is possible using normal-phase silica gel, reversed-phase layers will resolve individual members of these groups into sharp, often well-defined, zones. However, it is only possible to detect unsaturated acylglycerides and fatty acids on reversed-phase layers. This may initially be viewed as a limiting feature of the technique, but as the separation number even with HPTLC layers in one dimension is rarely more than 20, there is always only a finite length of chromatographic layer available in which the separation can occur. Hence, as the saturated lipids are undetectable, there is more separation capacity available for unsaturated compounds.

The reversed-phase separation of TG has resulted in a method for the identification of fatty oils. The protocol is given in the BP98 appendix XN and the EP97 (2.3.2) and shows a typical chromatogram obtained on HPTLC RP18 layers for a number of seed oils. The test method acts as an identitification for a wide range of oils as each has a TLC 'fingerprint' of unsaturated acylglycerides unique to itself. Solvent mixtures that give good separation reproducibility for reversed-phase are given in **Table 5**. Solvent mixtures 1 and 2 are comparable, but solvent mixture 3 gives similar resolution for the TG at lower R_F values and also good resolution for many of the DGs, MGs and FFAs. This solvent mixture therefore has been used effectively for investigations into the deterioration of frying oils. **Figure 4** shows a typical chromatogram of a blended frying oil.

As the separation has occurred almost purely by partition, it is possible to relate the positions on the chromatogram of the acylglycerides to the degree and the position of the unsaturation in the molecule. This then enables the prediction of the position of acylglycerides on the chromatogram and aids in the

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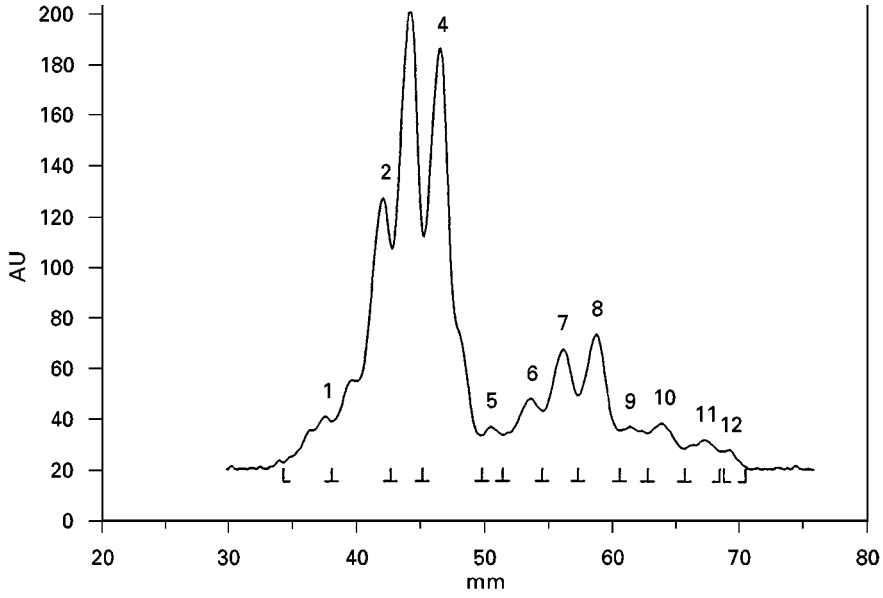


Figure 4 Separation of unsaturated acylglycerides and FFA on HPTLC silica gel RP18 glass plates. Mobile phase: dichloromethane–ethyl acetate–methanol–acetic acid (27 : 22 : 38 : 12, v/v). Detection with 1% w/v phosphomolybdic acid in ethanol. Plate heated to 100°C for 5 minutes. Scanned at 700 nm with a spectrodensitometer. Sample: degraded blended frying oil. Peaks 1–5 are triacylglycerides, peaks 6–8 are diacylglycerides, peak 10 is a free fatty acid and peaks 11 and 12 are monoacylglycerides.

identification of unknowns. The acylglycerides are separated according to the equivalent carbon number (ECN). This is defined as:

$$ECN = CN - 2n$$

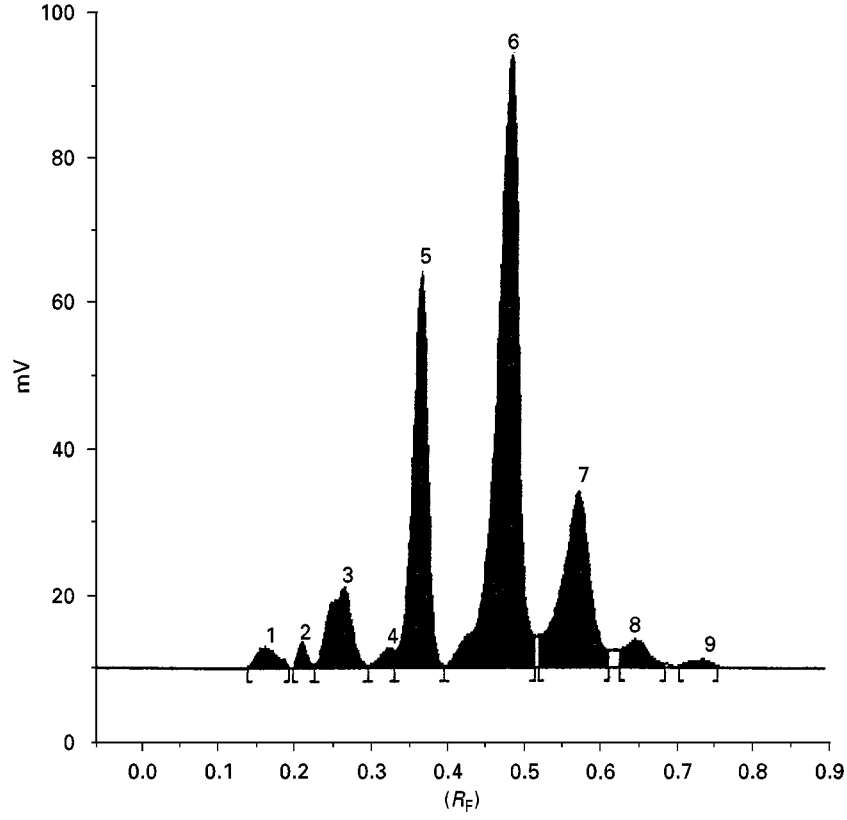
where CN = carbon number, n = number of double bonds.

However, this does not take into consideration the position of the double bonds. For this reason in HPLC an adjustment has been made to this equation:

$$ECN = CN - d_1n_1 - d_2n_2 - d_3n_3$$

where n_1, n_2, and n_3 are the number of double bonds attributable to oleic, linoleic and linolenic acids, respectively.

The values d_1, d_2 and d_3 are calculated by means of reference triacylglycerides. They are: $d_1 = 2.60$, $d_2 = 2.35$ and $d_3 = 2.17$.



Wavelength: 700 nm

Peak No.	R_F value	%
1	0.16	1.52
2	0.20	1.87
3	0.26	5.85
4	0.32	1.42
5	0.36	28.98
6	0.48	44.93
7	0.57	12.84
8	0.64	2.01
9	0.74	0.58

Figure 5 Separation of unsaturated triacylglycerides on an HPTLC silica gel RP18 layer impregnated with silver nitrate (5% w/v solution). Mobile phase: dichloromethane–ethyl acetate–methanol–water–acetic acid (25 : 20 : 35 : 6 : 6, v/v). Detection with 1% phosphomolybdic acid in ethanol. Plate heated to 130°C for 10 minutes. Scanned at 700 nm with a spectrodensitometer. Sample: Fresh blended frying oil. Peaks 3 and 6 are triolein and trilinolein respectively. Other peaks are other unsaturated triglycerides, sterols, and antioxidants unidentified.

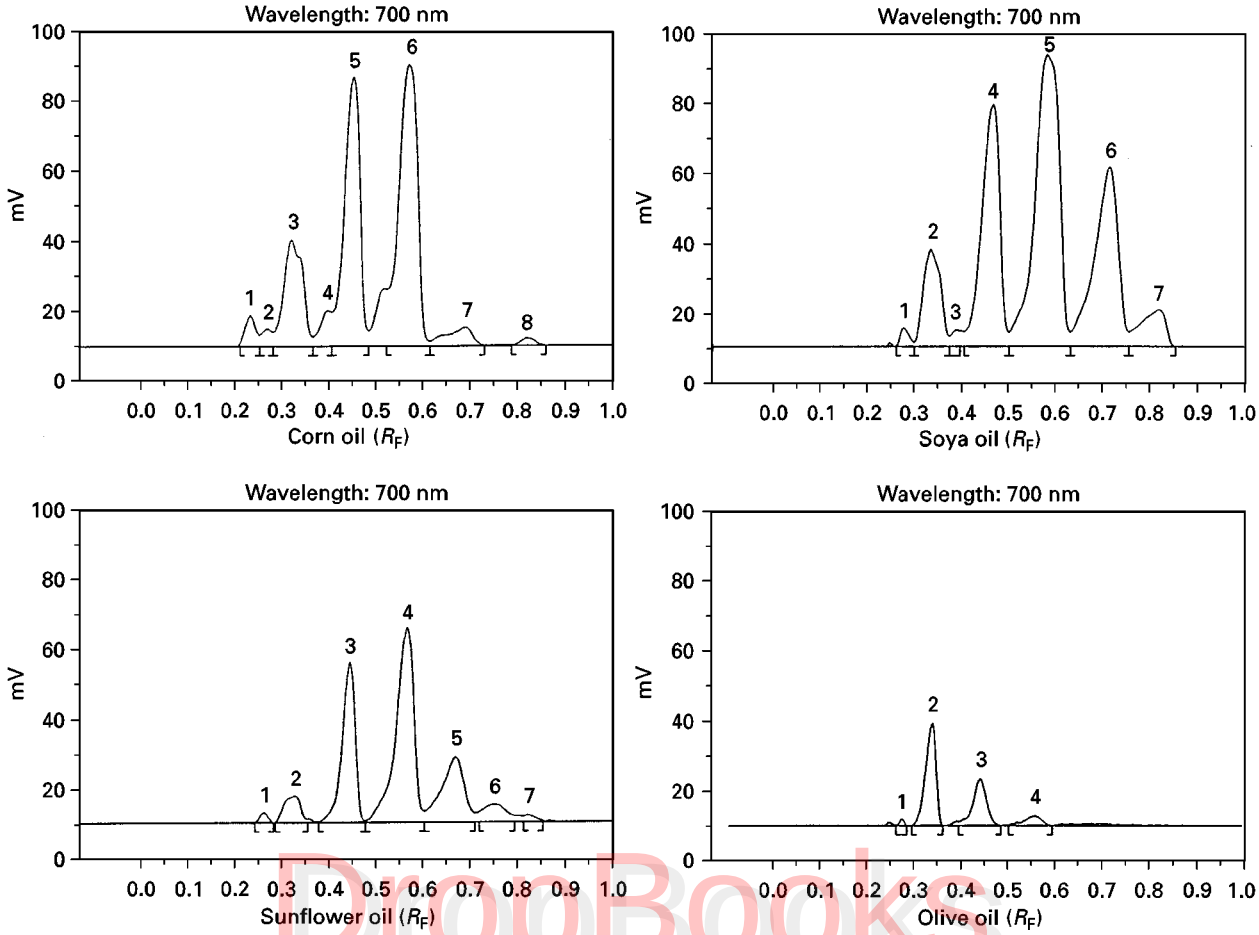


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Figure 6 Separation of unsaturated triacylglycerides on an HPTLC silica gel RP18 layer impregnated with silver nitrate (5% w/v solution). Separation conditions as in Figure 5. (Corn oil) Peaks 3 and 6 are triolein and trilinolein respectively. Other peaks not identified. (Soya oil) Peaks 2 and 5 are triolein and trilinolein respectively. Other peaks not identified. (Sunflower oil) Peaks 2 and 4 are triolein and trilinolein respectively. Other peaks not identified. (Olive oil) Peaks 2 and 4 are triolein and trilinolein respectively. Other peaks not identified.

Silver nitrate As with normal phase silica gel, it is possible to modify reversed phase silica gel with silver nitrate. Pre-coated reversed phase silica gel layers can either be impregnated or, where applicable, silver nitrate can be added to the mobile phase. A suitable impregnating reagent can be prepared with silver nitrate (5% w/v) dissolved in water–methanol (10 : 90, v/v). The separations obtained indicate a much stronger though limited resolving capability than is possible with unmodified reversed-phase layers (see **Figure 5**). The triacylglycerides are separated over a much wider R_F range enabling more marked differences to be observed in the chromatograms for a number of plant seed oils. A comparison of these is shown in **Figure 6**. However, as mentioned previously the technique does have limitations. DGs, MGs, and FFAs will all be found at or near the solvent front if the mobile phase has been adjusted to give maximum resolution for the triacylglycerides. Detection is also not as sensitive as for the corresponding reversed-phase layers (about a four-fold reduction).

Detection Methods

Detection of acylglycerides relies upon the use of chemical reagents as any UV absorbance is weak and none of these neutral lipids show any natural fluorescence (either in the visible or UV spectrum). Most chemical methods rely on reduction or charring techniques for acylglycerides. However, these can still be quite sensitive with the limit of detection usually being in the nanogram range. FFAs are much more reactive and hence a much wider range of detection reagents are available. The same applies to any degradation products due to oxidation where aldehydes, ketones or esters may have formed.

Detection on Normal Phase Layers

Visualization of both saturated and unsaturated acylglycerides is easily achieved on normal phase silica gel layers. However, it should be borne in mind that if the detection reagent is a charring one, then care must be taken when commercial pre-coated plates or sheets are used, particularly with sulfuric acid or chlorosulfonic acid. This is because in order to obtain good reproducibility and abrasive resistance, the pre-coated layers contain a small percentage of a polymeric organic binder. Unfortunately this can also char along with the sample components limiting the contrast between chromatographic zones and the background. However, if the temperature and duration of heating are carefully controlled, good results can be obtained.

Iodine vapour gives very good sensitivity, giving yellow-brown zones on a pale yellow background. The unsaturated compounds are stable for a much longer period of time than the saturated ones. The interaction with the π double bonds forms an iodine complex that is much more stable than the adsorption of iodine by the saturated compounds, which is reversible. These results can be made much more permanent by spraying the plate with soluble starch solution that forms dark blue complexes on the zones where iodine has been adsorbed.

Phosphomolybdic acid reagent (1–5%, w/v solution in ethanol) is probably the most popular reagent for lipid detection and gives a limit of sensitivity of 50–200 ng, depending on the glyceride. Zones appear after heating as blue-grey on a yellow background. This yellow background can be destained by exposure to ammonia vapour.

Other reagents that have been used to good effect are listed along with the above in **Table 6**.

Detection on Reversed-phase Layers

Of all the reagents listed in Table 6, the first four can also be used on reversed-phase layers. However, they can only be used to detect unsaturated acylglycerides and FFAs. Sensitivity, however, is on a par with normal phase layers with both iodine vapour and phosphomolybdic acid giving the best results. Charring reagents are best avoided as the background easily chars as well due to the fact that it is bonded with an aliphatic carbon chain.

Detection on Argentation-modified Phases

On commercial pre-coated layers, phosphomolybdic acid gives results comparable with those obtained on normal or reversed-phase plates. There is usually a lack of background staining which improves the contrast. The use of ammonia vapour though is not to be recommended as this reacts with the excess silver nitrate and a brown speckled background appears. For the reversed-phase plates, heating is required at a higher temperature (150°C for 10 minutes) to detect the unsaturated zones.

Some charring techniques have been used for 'home made' normal phase silver nitrate modified layers but these involve the use of very aggressive chemical reagents.

Table 6 Detection reagents suitable for the visualization of acylglycerides and free fatty acids on normal silica gel layers (non-commerical)

Detection reagent	*Acylglyceride detected*	*Observation*
Iodine vapour	Both saturated and unsaturated	Yellow/brown zones
Phosphomolybdic acid spray or dip followed by heating at 100–120°C for 10 minutes	Both saturated and unsaturated and FFA	Blue-grey zones on yellow background
Manganese (II) chloride/sulfuric acid [(0.2 g manganese chloride in water (30 mL) – methanol (30 mL) plus sulfuric acid (2 mL)] Heat at 100–120°C for 10 minutes	All acylglycerides and FFA	Brown zones on white background
Copper (II) acetate/sulfuric acid (copper acetate (3% w/v) in phosphoric acid (10% v/v)) Heat at 100–120°C for 10 minutes	All acylglycerides and FFA	Brown-grey zones on a white background
Sulfuric acid (10–20% v/v) Heat at 120–150°C for 15 minutes	Both saturated and unsaturated and FFA	Black or grey zones
Berberine solution (10 mg/100 mL ethanol)	All acylglycerides	Yellow fluorescent zones under UV at 360 nm

Future Developments

It seems unlikely that major developments will occur in the future with improvement of the separation method of acylglycerides on normal and silver nitrate impregnated silica gel. However, the use of the newer commercially available smaller particle size (~ 4 μm) and spherically shaped particles will result in an improvement in resolution, sensitivity and scanned peak shape of chromatographic zones. Automated multiple development (AMD) has already proved to be an excellent analytical tool for focusing zones in lipid separations but is still very much in its infancy with a big potential available for acylglyceride separations.

There is no doubt that reversed-phase HPTLC provides a reliable method for following the breakdown of oils and fats in use. The commercial possibilities here have yet to be fully exploited. There is still much work to be done in developing reliable, but simple, and rapid methods of analysis for triacylglyceride breakdown. Presently available HPTLC procedures not only have the potential to analyse and quantify the total FFA, but also to separate these and determine them individually. Some quantitative work on acylglycerides has already been accomplished, but in the future it should be possible to quantify far more. One of the present drawbacks has been the lack of availability of pure standards, particularly for many of the unsaturated acylglycerides. This is not altogether surprising as many are unstable and need to be kept deep frozen to avoid degradation.

The use of TLC for the analysis of triacylglycerides has further potential in the quantification of other organic species that may be present in oils and fats. Some oils naturally contain tocopherol, which acts as an antioxidant, and other oils may have this, or other antioxidants, added to extend their life. Sterols can also be present. As TLC requires little sample preparation before application to the chromatographic layer, the technique is usually quite easy (and many samples can be analysed at the same time). Many of these other compounds can be separated and determined quantitatively. The future of TLC for the analysis of triacylglycerides shows considerable potential.

See also: **II/Chromatography: Thin-Layer (Planar):** Densitometry and Image Analysis; Layers; Spray Reagents. **III/Impregnation Techniques: Thin-Layer (Planar) Chromatography. Lipids:** Gas Chromatography; Liquid Chromatography; Thin-Layer (Planar) Chromatography. **Oils, Fats and Waxes: Supercritical Fluid Chromatography. Silver Ion:** Liquid Chromatography; Thin-Layer (Planar) Chromatography. **Triglycerides:** Liquid Chromatography. **Appendix 17: Thin-Layer (Planar) Chromatography: Detection.**

Further Reading

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